Biotechnology Techniques Vol 2 No 1 63-68 (1988)
Received as revised February 15

DIRECT SCREENING FOR ACID PHOSPHATASE PRODUCTION ON BCIP-AGAR PLATES[1]

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SUMMARY

A direct screening procedure for detection of acid phosphatase activity was established using medium containing 5-bromo-4-chloro-3-indolyl phosphate. Production of acid phosphatase could be suppressed by adding phosphate. This technique will be useful for screening cell populations for acid phosphatase activity as well as studying its regulation.

INTRODUCTION

The acid phosphatase gene system has been a useful model for studying gene regulation in many organisms, including bacteria (Dassa, et al., 1982), yeast (Rogers, et al., 1982), Neurospora (Rodrigues and Rossi, 1985), and Aspergillus (Dorn, 1965; Caddick and Arst, 1986). The expression of phosphate-repressible acid phosphatase is regulated by the phosphate concentration in the medium; low levels stimulate the production of secreted acid phosphatase, while high levels suppress the synthesis of the enzyme. Detection of enzyme activity, however, has relied on standard assays of culture filtrates from liquid culture (Casida, 1959; Toh-e, et al., 1973) or replica plating techniques from solid media followed by colorimetric staining (Touati and Danchin, 1987). Both of these procedures are cumbersome and time consuming. Direct staining of agar

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plates by flooding with a colorimetric solution has been used for detection of acid phosphatase activity (Dorn, 1965), but this procedure may lead to mixing of populations, particularly with sporulating cultures.

The lactose analogue 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal) has been useful for direct screening of colonies for expression of β-galactosidase and its lacZ gene fusion products (Casadaban, et al., 1983; Hall, et al., 1983; van Gorcom et al., 1985). The chromogenically-similar substrate, 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) has been employed in cytochemical staining for alkaline phosphatases (Horwitz, et al., 1966; Kirkeby and Moe, 1987) and immunoblotting procedures using alkaline phosphatase and alkaline phosphatase-conjugated antibody (Blake, et al., 1984; Knecht and Dimond, 1984).

This paper describes the use of BCIP-containing culture media for direct screening of acid phosphatase expression in both Aspergillus ficuum isolates and Glycine max (soybean) suspension cultures.

MATERIAL AND METHODS

Strains. A. ficuum NRRL 3135 (SRRC 265) was obtained from Dr. Rudy Wodzinski (Dept. of Biological Sciences, Univ. of Central Florida, Orlando, FL) and A. ficuum isolate (SRRC 279) was obtained from the USDA Southern Regional Research Center collection. Spore suspensions prepared in sterile H_2O were used to inoculate plates. Hylon V starch medium was prepared as described previously (Gibson, 1987).

Suspension cultures of Glycine max (27C) were obtained from Dr. Jack Widholm (Dept. of Agronomy, Univ. of Illinois, Champaign-Urbana, IL) and maintained with weekly subculturing in the medium of Murashige and Skoog (1962) with 0.4 mg 2,4-dichlorophenoxyacetic acid/l (Murashige and Skoog, 1962).

Culture Medium. Modified M9 medium (van Gorcom et al., 1985), containing 1.5% agar, were adjusted to 0, 10 mM, 20 mM, 40 mM, 60 mM, 80 mM, and 100 mM phosphate with Na_2HPO_4 and KH_2PO_4. After autoclaving and cooling in a 68°C water bath, filter-sterilized solutions of 2 ml of 20 mg/ml BCIP (Bio-Rad Laboratories, Richmond, CA), 2 ml 1 M $MgSO_4$, 20 ml 50% glucose, and 1 ml 0.1 M $CaCl_2$ were added to each liter of media. For culturing of plant cells, 2 ml of 10 mg/ml

BCIP was used. Plates were maintained in a 30°C oven in darkness for up to 5 days.

RESULTS

Growth of cultures on BCIP-containing media. Aspergillus ficuum cultures grown on phosphate-deficient media containing BCIP began to appear blue within two days of growth (Fig. 1). Visual differences in staining intensity were apparent between the two A. ficuum isolates. The mycelium of isolate NRRL 3135 was more intensely blue stained than that of isolate SRRC 279. This difference in staining intensity was confirmed by production of enzyme in liquid culture under phosphate-limiting conditions. When grown on Hylon starch-containing medium (Gibson, 1987) isolate NRRL 3135 is a hyper-producer of extracellular acid phosphatase, while isolate SRRC 279 produces approximately 10% of the amount of acid phosphatase found with isolate NRRL 3135.

The soy suspension cultures which had been maintained in phosphate-deficient medium readily turned blue within four hours of culture. Suspension cells from phosphate-containing media turned light purple.

Phosphate repression of enzyme expression. Both isolates of A. ficuum grown on BCIP-containing medium with varying levels of phosphate exhibited a decreased blueness. At 80 to 100 mM phosphate, little or no blueness was evident. A more intense blue was present in the hyperproducing NRRL 3135 isolate at 20 to 60 mM phosphate, while the SRRC 279 isolate appeared to be lighter in color. A difference in color intensity between the two isolates was evident at 60 mM phosphate; isolate NRRL 3135 was blue while isolate SRRC 279 was unstained.

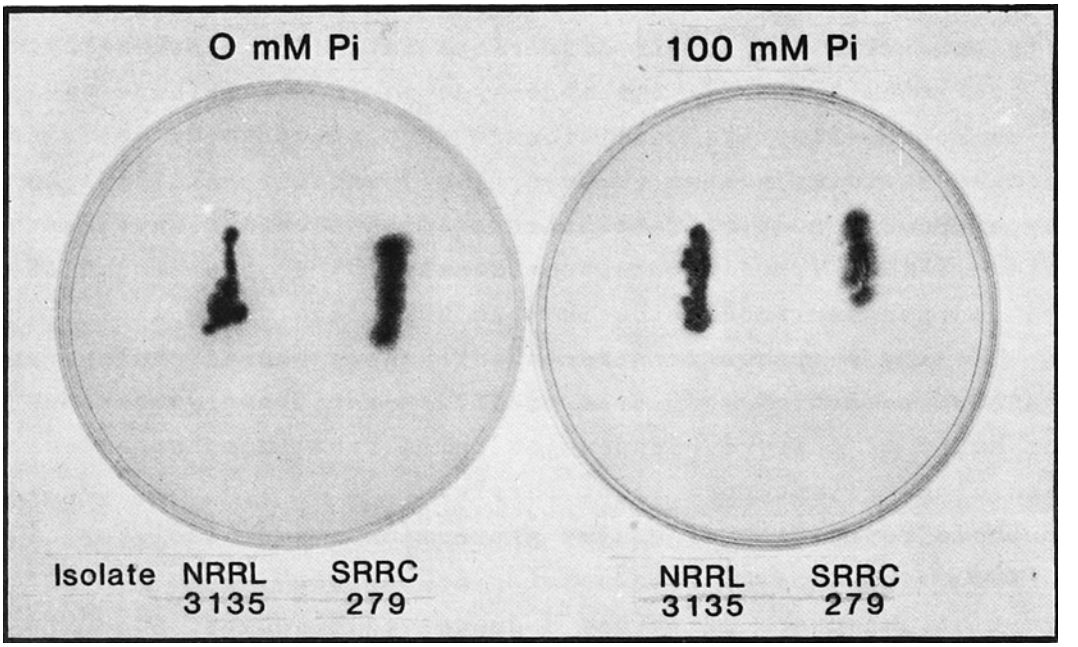

Figure 1. Aspergillus ficuum isolates NRRL 3135 and SRRC 279 after 48 hr growth at 30°C on BCIP-agar plates containing 0 mM Pi and 100 mM Pi.

DISCUSSION

The incorporation of BCIP (Xphos) in culture media may be a useful method for rapid screening of expression of acid phosphatase activity. The agar technique developed in this study would be useful in preliminary screening of mutants for loss of expression of acid phosphatase rather than culturing techniques in low and high phosphate media as described in Bergman et al., 1986, for detecting transformants with plasmids containing deletion mutations.

The medium described here might also be useful for the cloning of acid phosphatase genes. The BCIP-agar procedure would allow direct detection of enzyme activity from the growing colonies without the possibility of cross-contamination due to flooding of plates. BCIP-containing media in Tris buffers has been used in screening for the alkaline phosphatase genes in Escherichia coli (Guan, et al., 1983) and Bacillus licheniformis (Hulett, 1984; Hulett et al., 1985).

In this report, we have demonstrated the potential usefulness of BCIP-containing medium for rapid screening for acid phosphatase activity in both fungal and plant cell cultures. The BCIP-containing medium allows a rapid and direct visualization of acid phosphatase production, and the intensity of blue coloration can be qualitatively used to rapidly assess the level of production. The expression of acid phosphatase activity was phosphate-repressible and was visualized by a diminution of blue coloration of the colonies. This method should be useful in the screening procedures for cloning and regulation of acid phosphatase genes in general as well as for rapid screening of cell populations for hyperproducers of phosphatase.

ACKNOWLEDGEMENTS

We thank Ms. Jenni Hew for technical assistance.

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